6/2/93 Dr. Raja. Please follow the enclosed instructions & prepare a formal application. Deadline August 14, 1993

Prof George A. Hawke

CONFIDENTIAL

INTEGRINS AND CYTOKINES IN AGING- AND CANCER-INDUCED OSTEOPOROSIS

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Osteoporosis due to aging and cancer are pervasive disorders, which will affect almost all humans as they age, resulting in tremendous cost to the health care system and to the individuals' quality of life. Bone is a composite matrix of collagen, and other proteins and hydroxyapatite and certain ions. Living bone is metabolically active and it constantly remodels its matrix and mineral deposition along lines of mechanical stress. Bone loss results from disturbance in the balanced actions of osteoclasts, which resorb bone matrix, and osteoblasts, which deposit bone matrix. In osteoporosis, bone resorption increases: increased resorption is particularly prominent in the immediate postmenopausal period. As an effect of aging, osteoblasts loose vigor and cannot repair the defect caused by osteoclasts. Both abnormalities result in diminished total bone mass, which lead to fracture. In cases like multiple myeloma, there is increased osteoclastic activity resulting in osteoporosis and bone fractures, with or without increased calcium in blood.

Integrins are known to mediate cell-matrix interactions and initiate signal transduction cascades via protein kinases that culminate in altered gene expression. Cells express more than one integrin type receptors. A critical step in bone resorption is the attachment of osteoclasts to bone surface. Cell adhesion receptors such as integrins may play a vital role in this process. The integrin $\alpha V\beta 3$ has been implicated in this function and has been termed the osteoclast functional antigen. However, this integrin is found only in the ruffling membranes and not in the tight attachment sites of the podosome, which obviously is mediated by another integrin. Our studies have indicated osteoclasts express different types of integrin receptors, including some novel ones. No systematic studies have been conducted to identify the integrin profiles of both osteoclasts and osteoblasts. This is essentially due to lack of a good in vitro model system of human osteoclasts.

Factors such as interleukin-1ß (IL-11ß), tumor necrosis factor-ß (TNB-ß), and interleukin-6 (IL-6) positively influence the differentiation of osteoclasts. Monocytes / macrophages can also be induced to differentiate into osteoclast-like cells in vitro. Transforming growth factor-ß (TGF-ß), which is stored in the bone matrix, is known to induce expression of integrins. In multiple myeloma, plasma cells secrete IL-1ß and TNF-ß, which may activate osteoclasts or recruit monocytes to differentiate into osteoclasts. In prostate cancer, osteoblastic metastasis develop and bone formation occurs around the metastatic sites due to the production of unidentified growth factor(s) by the cancer cells. Normal individuals possess a natural antagonist to IL-1ß. Is this factor missing, diminished or non-functional in the patients with osteoporosis or multiple myeloma?

QUESTIONS OF INTEREST

(1) How do osteoclasts recognize and attach to the bone surface? How many types of integrins are involved in bone resorption? What are their relative contributions to the function of bone resorption?

(2) What are the integrin profiles of osteoclasts and osteoblasts? Are there any novel integrins expressed specifically in osteoblasts and osteoclasts?

(3) Are there other receptors types involved in this process?

(4) What are the steps involved in the signal transduction pathway that lead to bone resorption?

(5) What are the effects of various agents that modify the osteoclast function on the osteoclast integrin profile?

(6) Is there a correlation between the osteoclast activity and the severity of multiple myeloma? What are the factors involved in the bone resorption activity in multiple myeloma?

(7) Is there a reliable in vitro test system to address these important questions?

(8) What is the status of IL-1 antagonist in patients with osteoporosis and multiple myeloma?

(9) Can one develop a gene therapy protocol armed with answers for the above questions to regulate the process of bone resorption?

Source: https://www.industrydocuments.ucsf.edu/docs/npjc0000

OBJECTIVES

(1) To identify the various integrin subunits expressed on osteoclasts and osteoblasts and elucidate their role in bone metabolism. (One of us [R.R.] is currently collaborating in these studies with Dr. Chandrika Kumar of Smith Kline Beecham, Molecular Genetics Division, King of Prussia, P.A.)

(2) To initiate and characterize permanent human osteoclast and osteoblast cell lines as in vitro test systems and determine the pharmacological effects of various factors that modulate osteoclast and osteoblast function using this in vitro assay system. The factors may include osteocalcin, parathyroid hormone (PTH), 1,25-OH_2vitamin-D_3, IL-1, IL-6, NaF, steroids, estrogens, androgens, biphosphonates, calcitonin, and others. To address the question of the effect of these factors on integrin expression in these cells.

(3) To inquire if peripheral monocytes from patients with osteoporosis and multiple myeloma are functionally different from those of normal individuals.

(4) To investigate the possible presence of genetic differences (eg. IL-1 antagonist) between individuals who do and do not develop osteoporosis?

(5) To characterize the mechanisms of increased osteoclast activity in patients with multiple myeloma using the in vitro assay system and design methods to decrease the activity under experimental conditions.

SIGNIFICANCE:

(1) The proposed studies will reveal the functions of various integrins present in osteoclasts and osteoblasts in relation to bone physiology. This information would be important for characterizing different disorders involving osteoporosis and help determine the course of therapeutic approach.

(2) Establishment of osteoclast and osteoblast cell lines would be invaluable to test different therapeutic agents in vitro.

(3) The above studies shall pave way for future studies on induction or suppression of osteoclast or osteoblast function using different agents such as calcitonin, parathyroid hormones, 1,25-dihydroxyvitamin-D3, and cytokines and other factors including novel synthetic peptides.

(4) Studies on monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma will help understand the severity of the disease in relation to the osteoporosis exhibited by MM patients.

(5) The results obtained from these studies would be of great diagnostic and therapeutic value. Most importantly, there is a great potential for gene therapy in osteoporosis and related disorders such as multiple myeloma, and Paget's disease.

A nucleus of Physician-Scientists at Dalhousie University has the necessary expertise in integrin technology, tissue culture, and clinical experience to carry out these studies. The division of Endocrinology and Metabolism is in the process of establishing an osteoporosis clinic. We are also evaluating assays for osteocalcin (a marker for bone formation) and for pyridoniline cross-links (a marker for bone resorption). Using the approaches stated above, we should be able to obtain answers for most of these questions within the target time. Described below is a budget for three years to carry out this project.

BUDGET

	1993-94	1994-95	1995-96
a. Salary for 3 technicians	$ 87,609	$ 90,237	$ 92,943
b. Graduate student	$ 15,295	$ 15,295	$ 15,295
c. Equipment purchase	$ 30,000		
d. Materials, supplies & incidentals	$ 120,000	$ 130,000	$ 135,000
Total:	**$ 252,904**	**$ 235,532**	**$ 243,238**

Source: https://www.industrydocuments.ucsf.edu/docs/npjc0000